Assessment of Metabolism in the Regenerating Mouse Liver by ^{31}P MRSI

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Introduction: In patients with hepatic metastases from colorectal cancer, resection of the tumor-containing portion of the liver is the only curative therapy. There is much interest in further improving the chance of long-term survival by administering adjuvant treatments such as cytotoxic chemotherapy, anti-antiogenic agents and oncolytic viral therapy after partial hepatectomy. We have previously shown in a rodent model that high-energy phosphates (NTP) detected non-invasively by ^{31}P magnetic resonance spectroscopic imaging (MRSI) decreased following partial hepatectomy (PH), and correlated with increased DNA synthesis. Furthermore, waiting for NTP recovery before administering cytoxic chemotherapy is necessary for survival [1]. The purpose of the current study was to measure levels of ^{31}P metabolites in mouse liver following PH to determine whether, similarly to findings in the rat, ^{31}P metabolites were non-invasive markers of regenerative status and readiness for further treatment. The small size of the mouse liver presents a challenge due to the decreased tissue volume for study. In mouse, voxel sizes on the order of 64 mm^3 are necessary which is an order of magnitude lower than voxel volumes used in rats. This was addressed by using a higher field strength magnet (7T), a smaller surface coil, and, in the second phase of the current study, adding proton-decoupling and weighted k-space sampling.

Methods: Partial (70%) hepatectomy was performed as described previously [2]. Mice undergoing sham-laparotomy and nonoperated controls were also studied. In *phase 1* of the experiment, ^{31}P MRSI was performed in separate cohorts at 24h (N = 8), 48h (N = 4), 72h (N = 4), and 96h (N = 8) after PH with rectangular k-space sampling, while in *phase 2*, 6 mice were studied 36h after PH using proton-decoupling and weighted k-space acquisition. All MR experiments were performed on a 7T Bruker spectrometer. Mice were anesthetized with isoflurane and placed prone over a 16mm diameter, two-turn ^{31}P surface coil. A sphere containing methylene diphosphonic acid (MDP) was placed in the center of the coil and used for flip angle calibration and quantitation. The ^{31}P coil platform was positioned inside a proton-tuned birdcage resonator which was used to obtain axial and sagittal proton images and, in *phase 2*, to proton-decouple. ^{31}P 3D-MRSI measurements were performed using a pulse-and-acquire sequence with hard-pulse width calibrated to obtain a 45° pulse at the liver, SW = 20000 and 1024 points sampled. In *phase 1*, an 8x8x8 rectangular matrix was acquired with TR=700ms, NEX=10. TR = 700 ms was chosen to increase sampling efficiency for NTP which has a short T1 in the liver. FOV varied depending on liver volume; voxel sizes ranged from 45-90 mm^3 with the smallest voxels used in the 24h PH mice. *Phase 2* employed Waltz-16 proton-decoupling, TR = 1.5s, FOV = 32 mm, and 13 phase encoding steps per direction with weighted averaging to generate a Hamming function with effective spatial resolution of 4x4x4mm^3. Total acquisition time was approximately 60 min in both phases. *Data Processing:* Registration of MRSI data to the anatomy was done with 3D Interactive Chemical Shift imaging (http://mrs.cpmc.columbia.edu/3dicsi.html). MRSI spectra were analyzed using jMRUI (http://www.mrui.uab.es/mrui/) and quantified after corrections for voxel volume, B1, saturation and flip angle [3]. Liver volumes were measured using ImageJ (http://rsbweb.nih.gov/ij/).

Results and Discussion: *Phase 1:* Fig. 1 shows normalized NTP levels vs. time after PH. Mean NTP at 24h after PH was lower than controls but not significant. Following phase 1, to improve resolution within the PME region along with SNR, the addition of proton decoupling and weighted k-space sampling was added during phase 2. *Phase 2:* Fig. 2a illustrates a representative cross-sectional weighted k-space MRSI grid superimposed on the MRI for a mouse 36h post PH. The image/grid is oriented with the mouse's anterior at the top. Each MRSI voxel measures 4x4x4 mm (64mm^3). The highlighted voxel has been expanded in Fig. 2b. Mean NTP was lower in mice 36h after PH than in controls, but was not significant (Fig 3). The mean phosphocholine (PC) component of the phosphomonester (PME) peak showed a decrease 36h post PH, however this not significant (P=0.23). Fig. 4 show liver volumes at baseline, 24, 36 and 48h after PH. As expected, liver volumes 24h post PH were much reduced but significaltly increased by 48h due to regeneration. In humans we have previously shown increased PE and decreased NTP following PH [4], while in rats, a significant decrease in NTP at 48h was noted [1]. In mice, peak DNA synthesis after 70% PH has been found between 24 and 48h [2]. The non-significant trend toward reduced NTP in both phases of this study suggests two possible reasons. First, experimental uncertainty may not have allowed us to detect changes in NTP. In phase 1, voxel sizes as small as 45 mm^3 were used at 24h post PH without weighted k-space sampling, resulting in sub-optimal SNR. However, with weighted k-space sampling at 36h PH, a significant change in NTP was still not detectable. Inter-subject variability in regenerative status could also result in a spread in the measured values. Second, the rate of DNA synthesis may not have exacted a cost in NTP that was detectable. Changes in PME and/or PE, PC were not observed in either phase of the study. This is similar to our finding in rodents, but unlike the human data. Further work is needed to understand why membrane phospholipid metabolism changes are not observed *in vivo* in rodents after PH.

Fig. 1

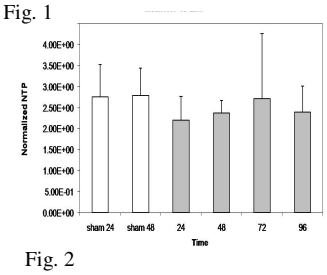
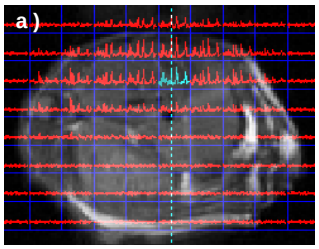


Fig. 2



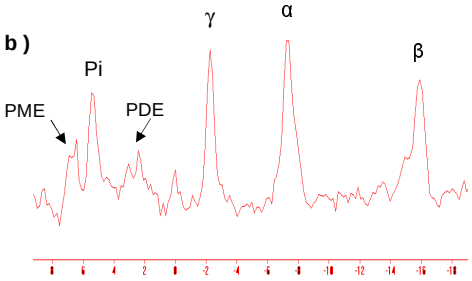


Fig. 3

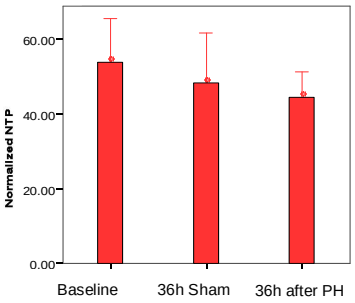
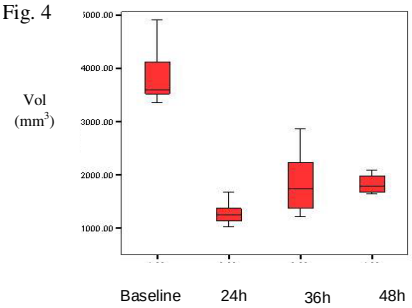


Fig. 4



Legends: Fig. 1: Mean NTP levels at various time points after 70% PH in mice measured by ^{31}P MRSI (*Phase 1* data). Fig. 2: *a)* Proton decoupled, acquisition weighted 3D ^{31}P MRSI overlying corresponding ^{1}H axial image of the liver (*Phase 2* data); *b* Expanded voxel highlighted in *a)*. Fig. 3: Mean NTP levels in mice 36h after PH. Note: Not comparable to Fig. 1 due to different sampling scheme. Fig. 4: Liver volumes (mm^3) measured after 24, 36 and 48h PH compared to baseline.

References: [1] Kooby *et al. Cancer Res* 60: 3800-3806, 2000 [2] Delman *et al. Hepatology* 39, No. 6, 2004 [3] Zakian *et al. Magn Reson Imaging.*18(2):181-7, 2000 [4] Zakian *et al. Magn Reson Med.* 54(2):264-71, 2005

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